IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Richard L. Moss, et al.
Application No.:	10/748,354
For:	TRANSGENIC MODEL FOR MYOCARDIAL FUNCTION
Filed:	December 30, 2003
Group Art Unit:	1632
Examiner:	SGAGIAS, Magdalene K.
Attorney Docket No.:	054030-0045

AFFIDAVIT UNDER RULE 1.132

I, Richard L. Moss, the undersigned, declare as follows:

All statements made herein are true to the best of my knowledge, or if made upon information and belief are believed to be true.

In response to the Office Action dated January 10, 2006 I supplied an affidavit showing the production of a transgenic mouse according to the specification of my patent application 10/748,354. As part of my submission, I provided genomic digests confirming the genotyping of the S342G transgenic mouse as disclosed in my patent application (Figures 1A and 1B). I also provided graphs showing the Rate of Force Redevelopment as a function of calcium concentration and Normalized Force as a function of time (Figures 2A and B) as disclosed in my patent application. In addition, I submitted tables (Table A and Table B) showing data developed in my lab comparing the absolute force generated by the same, S342G mutant (Table A) and the change in heart rate of the transgenic animals (Table B). Unfortunately, the legends for Figures 1A and 1B and Figures 2A and 2B refer to the transgenic animal conforming to the naming convention used in the art in which the myosin's amino acid residues are standardized to chicken pectoralis myosin (e.g. as an S341G mutant). This naming convention is described in the patent application at, for example, page 15, line 20, page 19-20, lines 14-5, page 39, lines 1-5 and in the alignments provided in the specification at pages 59-60. In order to clarify any confusion regarding the data provided to the Office in my declaration dated July 10, 2006, I

attach hereto revised figures referring to the transgenic animal numbering the amino acid residues based on mouse myosin peptide residues alone as is recited in the patent claims.

Accordingly, the successful production of a S342G transgenic mouse by the methods described in this patent application is commensurate with the data derived from the transgenic mouse prepared as described in the application.

This declaration is made with the knowledge that willful false statements and the like are punishable by fine or imprisonment, or both under 18 USC Sec. 1001, and may jeopardize the validity of the subject patent application or ay patent issuing thereon.

Dated: Dec 4, 2006

By: Richard L. Moss
Richard L. Moss

Figure 1.

S342G KO PCR

This photograph represents results obtained from PCR-based genotyping designed to identify the mutated S342G gene in our transgenic mice. The white band in each column is mutant DNA, amplified out of our S342G transgenic mice. There is no band in the lane for the 13th sample because it is a negative water control and run only to check that reagents are not contaminated. There are multiple white bands in the first lane because it is a DNA standard, and does not represent DNA from our colony. Every other column has one white band in it, signifying that each of these animals carries our mutant gene.

S342G WT PCR

This image represents results obtained from a second PCR-based genotyping experiment on the same 13 samples as the KO PCR. This experiment was designed to identify the healthy endogenous S342G gene, also called a non-transgenic gene, in our mice. There are no bands in any lane except the positive control lane (DNA we know does carry the gene, and meant to test that the reagents are working). In other words, these two pictures, when taken together, show that we have bred the S342G heterozygous mouse to create the S342G homozygote mutant strain. These animals carry two copies of our mutant S342G DNA and no healthy, wild-type DNA, meaning they are targeted knock-out mice at the S342G locus.

Figures 2A and **B.**

Are graphs plotting the rate of force redevelopment (s^{-1}) as a function of calcium potential (pCa) and normalized force as a function of time. These experiments were performed to assess the turnover kinetics of the mutant myosin by determining the rate of force development in myocardium from the S342G transgenic mice. The experiment employs the following protocol: permeabilized myocardium is activated to varying degrees by varying the amount of activating Ca2+ in the muscle bath; once steady force is achieved, the preparation is rapidly released and restretched, thereby reducing force to zero; the rate constant of force redevelopment (k_{tr}), which is a direct measure of myosin turnover kinetics, is then estimated by fitting an exponential to the time course of force redevelopment (Figure 2B). As disclosed in the present specification, the substitution at residue 342 of myosin heavy chain slowed the rate of rise of force approximately 30% (Figure 2A), indicating that this residue is a primary determinant of myosin turnover kinetics in the heart. Furthermore, the inventors' previous finding that mouse is unique in having a serine at residue 342 of cardiac myosin and that all other myosins in other species have a glycine at 342 indicates that this substitution is responsible for the faster turnover kinetics of mouse cardiac myosin.

Table A.

Is a table showing the absolute force generated by the S342G transgenic and the wild type control.

Table B.

Is a table showing the heart rate of the S342G transgenic and the wild type control.

Figure 1A

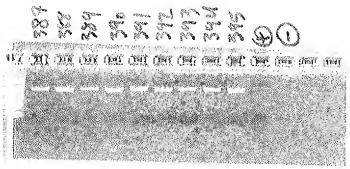

Figure 1B

Figure 2A

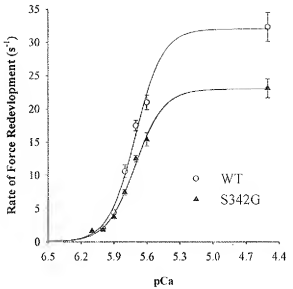

Figure 2B

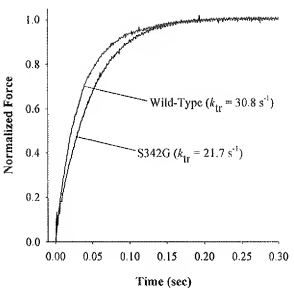

Table A

Animal	Force (mN/mm^2)
Wild Type	32±5 (n=12)
S342G Transgenic	23±2 (n=8)

Table B

Animal	Heart Rate (beat/min)
Wild Type	606
S342G Transgenic	550

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